Short Communication

Impact of ultrasonic irradiation pretreatment of water-soluble cellulose on enzymatic hydrolysis

Ryo-ichi Nakayama[1] · Momoko Murakami[1] · Norikazu Namiki[1] · Masanao Imai[2]

Received: 10 December 2019 / Accepted: 26 March 2020 / Published online: 7 April 2020

Abstract

This study investigated the effects of ultrasonic irradiation on the enzymatic hydrolysis of water-soluble cellulose. Two types of cellulases (from *Aspergillus niger* and *Trichoderma reesei*) were employed for the enzymatic hydrolysis of carboxymethyl cellulose (CMC). An assay of the enzymatic hydrolysis of water-soluble cellulose was evaluated based on the production of glucose. The optimum set of operating conditions for ultrasonic irradiation involved an ultrasonic irradiation power and time of 116 W and 30 min, respectively. The initial reaction rate of enzymatic hydrolysis of CMC was increased with ultrasonic pretreatment (116 W) compared to that of the ultrasonication-free system. The kinetic parameters (Michaelis–Menten constant; K_m and maximum reaction rate; V_{max}) were examined to evaluate the reactivity. As a result of ultrasonic irradiation, the value of K_m was decreased to 0.2 times its original value in the system employing *A. niger*, and to 0.6 times its original value in the system employing *T. reesei*. Ultrasonication increased the value of V_{max} to 1.3 times its original value for both cellulases. Moreover, the viscosity of the CMC solution was decreased by ultrasonic irradiation, decreasing the activation energy of the reaction and increasing its frequency factor. Ultrasonic irradiation can therefore be employed in process design to control viscosity and achieve high-performance enzymatic hydrolysis of water-soluble cellulose. Ultrasonication is an effective application for accelerating enzymatic hydrolysis of cellulose and design lower energy industrial processes for cellulose degradation.

Keywords Ultrasonic irradiation · Carboxymethyl cellulose · Viscosity · Enzymatic hydrolysis · Cellulase

List of symbols

A	Frequency factor (Pa s)
c	CMC concentration for the measurement of viscosity (g/L)
C	Glucose concentration (mol/L)
E	Activation energy (J/mol)
K_m	Michaelis constant (g/L)
I_r	Ultrasonic irradiation power (W)
MW_{app}	Apparent mean molecular weight of CMC (g/mol)
r	Reaction rate (mol/L s)
R	Gas constant [J/(mol K)] = 8.3 [J/(mol K)]
T	Temperature (K)
V_i	Initial reaction rate (mol/L s)
V_{max}	Maximum reaction rate (mol/L s)

Subscripts

0	Initial concentration, used in $[E_0]$ (g/L) and $[S_0]$ (g/L) for the enzymes and the CMC solution, respectively

Greek symbols

μ	Viscosity of sample (Pa s)
$\varnothing$	Diameter of ultrasonic device (cylindrical shape) (cm)

✉ Ryo-ichi Nakayama, bionakayama.ryo@cc.kogakuin.ac.jp | [1]Department of Environmental Chemistry and Chemical Engineering, School of Advanced Engineering, Kogakuin University, 2665-1 Nakano-machi, Hachioji, Tokyo 192-0015, Japan. [2]Course in Bioresource Utilization Sciences, Graduate School of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa Prefecture 252-0880, Japan.

SN Applied Sciences (2020) 2:846 | https://doi.org/10.1007/s42452-020-2606-6

1 Introduction

Lignocellulosic biomass is an abundant carbohydrate source on earth. Cellulose and hemicellulose comprise up to two-thirds of the lignocellulosic biomass [1–3]. Cellulosic is strategically important naturally occurring polymer with significant applications in food and biotechnology processes. Cellulose is a long-chain polymer of glucose monomers linked by β-1,4 glyosidic bonds. Cellulose from different sources may be differing in the crystalline structures and binding characteristics. Carboxymethyl cellulose (CMC) is the model compound most commonly used in recent research because it has a generally amorphous structure.

Carboxymethyl cellulose (CMC) is a common water-soluble cellulose derivative. It has been widely used in the food and cosmetic industries as a viscosity modifier and in the mineral industry as a modifier in flotation processes. Furthermore, in the biomedical field, it has been employed for the prevention of post-operative adhesions and epidural scarring [4–6]. For use in these applications, CMC needs to be depolymerized to improve or modify its physical properties. The physical properties of polysaccharides such as CMC depend on their molecular weight and viscosity. It is therefore important to develop effective methods for obtaining polymers with the lower molecular weight and viscosity. The enzymatic decomposition of polymers has been widely studied. However, conventional enzymatic methods have a slow reaction rate. Recently, the use of ultrasonication in enzymatic reactions has been extensively studied, and ultrasound has also shown significant applications in food and biotechnology processes.

Ultrasound is a mechanical wave with a frequency between 10 kHz and 20 MHz [7]. It can impart high impart high energy to a reaction by cavitation, which can improve the reaction in several ways. Ultrasonication is expected to have the following benefits for enzymatic reactions; (1) controlling of the viscosity of polymer solutions; (2) avoiding harmful products that are not conducive to enzymatic reaction; (3) avoiding the use of chemical reagents that may pollute the environment or corrode the equipment; and (4) full utilization of the reaction materials, which avoids waste or pollution [8].

The main object of this work was to clarify the effects of ultrasonic irradiation on the viscosity and the enzymatic depolymerization of high-molecular weight CMC. We examined the viscosity of CMC and the reaction rate of enzymatic hydrolysis under different ultrasonic irradiation power levels. The reactivity of the enzymatic hydrolysis of cellulose pretreated by ultrasonic irradiation was evaluated using kinetic data.

2 Experiments

2.1 Materials

CMC and two kinds of cellulases (from *Aspergillus niger* and *Trichoderma reesei*) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Acetic acid, sodium acetate, and sodium chloride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Methods

2.2.1 Enzymatic hydrolysis of water-soluble cellulose with ultrasonication

The initial concentrations of the CMC solutions ($[S_0]$) were 2.5–10 g/L. A volume of 100 mL of each concentration was prepared. The initial concentration of the cellulase solution ($[E_0]$) was 5 g/L, and a volume of 10 mL was prepared. Both solutions were prepared using a 0.2 mol/L-acetate buffer (pH = 4.8). Ultrasonic irradiation at a frequency of 24 kHz was directly delivered to the CMC solution using a horn-shaped cylindrical device (UP200S, Hielscher Ultrasonic GmbH, Germany). The cross-sectional area of the ultrasonic horn was 153 mm^2. The guaranteed maximum surface power intensity was 1.3 W/mm^2. The output was controlled at 20%, 40%, or 60% of the maximum output. The applied ultrasonic irradiation power (I_r) was therefore 39 W, 77 W, or 116 W. The cylindrical radiating tip [diameter (φ) = 14 mm] was placed into the CMC solution, which was cooled to 283 ± 2 K.

The CMC solution was irradiated without enzyme for 30 min. After ultrasonic irradiation, the enzymatic reaction was initiated by adding the cellulase solution to the CMC solution at 310 K. The enzymatic reaction was conducted in the absence of ultrasonic irradiation. Samples (20 µL) were taken from the reaction solution and immediately heated by immersion in boiling water for 1 min to stop further enzymatic reaction by heat inactivation. The sampling time was determined as the time when the heat inactivation of the enzyme was complete.

The reaction progress was determined based on the production of glucose in the CMC solution. The mutarotase–glucose oxidase method was employed to determine the glucose concentration. The glucose concentration was examined calorimetrically using Glucose C2-Test Wako (purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan), a special reagent kit for quantitatively analyzing glucose. The absorbance was measured using a spectrophotometer (505 nm; UV mini-1240, Shimadzu, Kyoto, Japan).

2.2.2 Measurement of the viscosity of the water-soluble cellulose solution

The concentrations of the CMC solutions were in the range 2.5–10 g/L (100 mL). The solutions were prepared using a 0.2 mol/L-acetate buffer (pH = 4.8). The ultrasonic irradiation power applied into the CMC solution was 39 W, 77 W, or 116 W. The CMC solution was then cooled to 283 ± 2 K. The CMC solution was irradiated for 30 min. After ultrasonic irradiation, the viscosity of the CMC solution was measured with a tuning-fork vibro-viscometer (SV-1A, A&D Co., Ltd., Japan) over a temperature range of 283–333 K.

The mean molecular weight of CMC was determined from the intrinsic viscosity ([μ]). Three different empirical equations (Huggins's equation and Mead–Fuoss equation) were employed to measure the intrinsic viscosity.

Huggins's equation was also employed for the analysis of the intrinsic viscosity [μ]:

$$\frac{\mu_{sp}}{c} = [\mu] + k'[\mu]^2 c \quad (1)$$

Mead–Fuoss equation was also employed for the analysis of the intrinsic viscosity [μ]:

$$\frac{(\ln \mu_{rel})}{c} = [\mu] - \beta[\mu]^2 c \quad (2)$$

The intersection on the ordinate indicated the intrinsic viscosity ([μ]) of CMC.

3 Results and discussion

3.1 Influence of the enzymatic hydrolysis of water-soluble cellulose by ultrasonication

Figure 1 shows the time course of glucose production by enzymatic hydrolysis of CMC with ultrasonication using the two kinds of cellulases. The measurements were carried out in triplicate, and the figure presents the arithmetical mean values. The glucose production of the system employing *A. niger* increased more rapidly than that of the system employing *T. reesei*. This could be explained by the different hydrolysis reaction mechanisms acting on the cellulose molecular chains [9]. The cellulase from *A. niger* contains more *β*-glucosidase, which hydrolyzes oligosaccharides to glucose. An assay of the enzymatic hydrolysis of CMC was evaluated based on the production of glucose. As a result, it was speculated that glucose production in the system employing *A. niger* would be more effectively enhanced than in the system employing *T. reesei*.

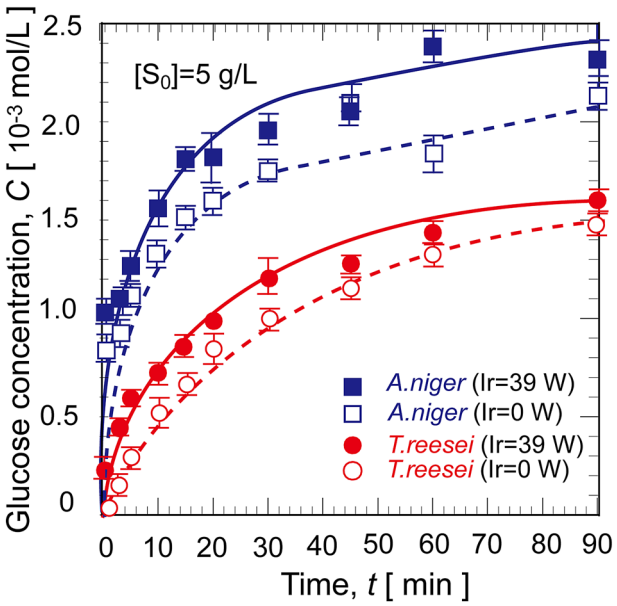

Fig. 1 Typical time course of enzymatic hydrolysis of CMC with ultrasonication.[S_0] = 5 g/L, [E_0] = 5 g/L, I_r = 39 W, irradiation time = 1800 s

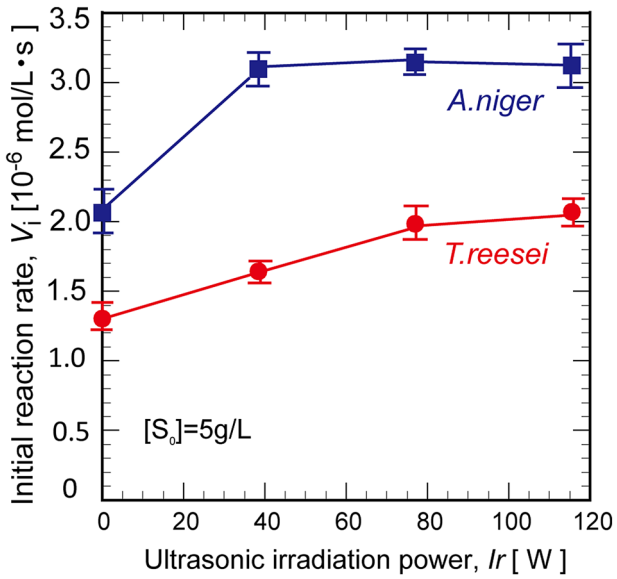

Fig. 2 Effect of ultrasonic irradiation power on the initial reaction rate using two different types of cellulase. [S_0] = 5 g/L, [E_0] = 5 g/L, irradiation time = 1800 s

Figure 2 shows the effect on the initial reaction rate (V_i) of changing the ultrasonic irradiation power before the enzyme reaction. When *A. niger* and *T. reesei* were used alone, V_i increased with increasing ultrasonic irradiation power. Figure 3 shows the effect of the ultrasonic power on the kinetic parameters. The maximum

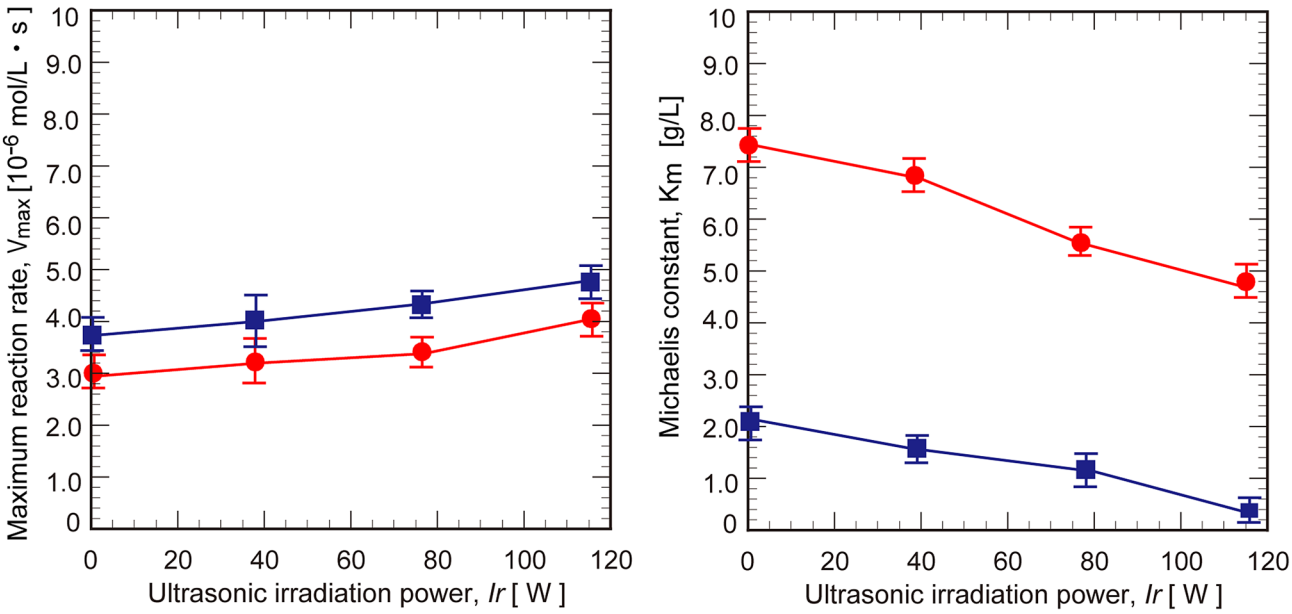

Fig. 3 Relationship between the kinetic parameters (V_{max} and K_m) and the ultrasonic irradiation power. (filled square) from *A. niger.* (filled circle) from *T. reesei*

reaction rate (V_{max}) and the Michaelis constant (K_m) for glucose production were calculated using Hanes –Woolf plot as follows. The value of $[S_0]/Vi$ was plotted against $[S_0]$ for reactions using the two cellulases (from *A. niger* and *T. reesei*) and ultrasonic irradiation pretreatment. According to Eq. (3), the intersection of the vertical axis indicates K_m/V_{max}, and the slope of correlated line presents $1/V_{max}$.

$$\frac{[S_0]}{Vi} = \frac{1}{V_{max}} \cdot [S_0] + \frac{K_m}{V_{max}} \tag{3}$$

For both cellulases, K_m decreased with increasing ultrasonic irradiation power. This is probably due to the intense pressure or shear forces that are generated as a result of cavitation. Exposure of the active site causes the enzyme to combine with the substrate and increase the reaction rate. These explanations are based on the different hydrolysis reaction mechanisms acting on the cellulose molecular chains. The value of V_{max} was slightly increased. With an increase in the ultrasonic power, the amount of cavitation increases, producing a strong physical effect on the activation of the adsorption mechanism. Ultrasonic waves expand the separation between the network chains of the CMC molecules in the solution and enhance the access of the enzyme to the substrate. A loosening of the polymer network in the dissolved substrate molecules increases the effective steric clearance per unit substrate that is available for the enzyme to act.

3.2 Influence of the viscosity of water-soluble cellulose solution

Figure 4 shows the effect of the concentration of the CMC solution on its viscosity, with ultrasonic irradiation powers of 39 W, 77 W, or 116 W for 30 min. To obtain the mean molecular weight of CMC, the arithmetic mean value of

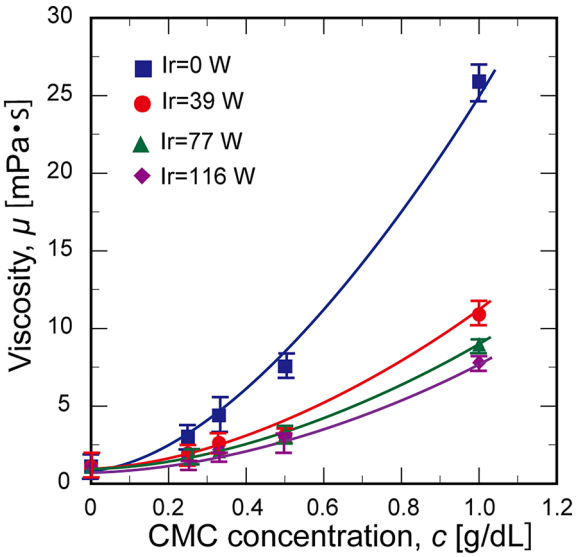

Fig. 4 Effect of the CMC concentration on its viscosity with ultrasonication. Concentration = 2.5 to 10 g/L, I_r = 0 to 116 W, irradiation time = 1800 s

the intrinsic viscosity is substituted into the Mark–Houwink–Sakurada equation.

The Mark–Houwink–Sakurada equation was employed using the empirical parameters $K = 1.23 \times 10^{-2}$ and $\alpha = 0.91$ [10].

$$[\mu] = K(MW)^{\alpha} \quad (4)$$

Figure 5 shows the apparent mean molecular weight of CMC (MW_{app}), which decreases with increasing ultrasonic irradiation power. We hypothesize that the CMC molecules do not decompose upon ultrasonication because the ultrasound output energy is only 70–209 kJ, which is lower than that of a covalent bond [11]. Instead, a decrease in the apparent mean molecular volume of CMC suggests that the networks of CMC molecular chains were weakened by cavitation due to ultrasonic irradiation.

Measurements were carried out in triplicate, and the arithmetical mean values are plotted in Fig. 6. The viscosity of the solution increased with increasing CMC concentration both with and without ultrasonication. A previous report suggested that it is difficult to produce cavitation in these solutions because of the existence of strong cohesive forces [12]. It has been reported that the viscosity of a carboxymethyl cellulose and polyvinyl alcohol solution decreases with increasing polymer concentration [13]. In the case of a high CMC concentration (10 g/L), the viscosity of the solution with ultrasonic irradiation (116 W) was 0.3 times that of the non-irradiated solution. Ultrasonic irradiation therefore effectively reduces the viscosity of CMC. With increasing ultrasonic irradiation power, the number

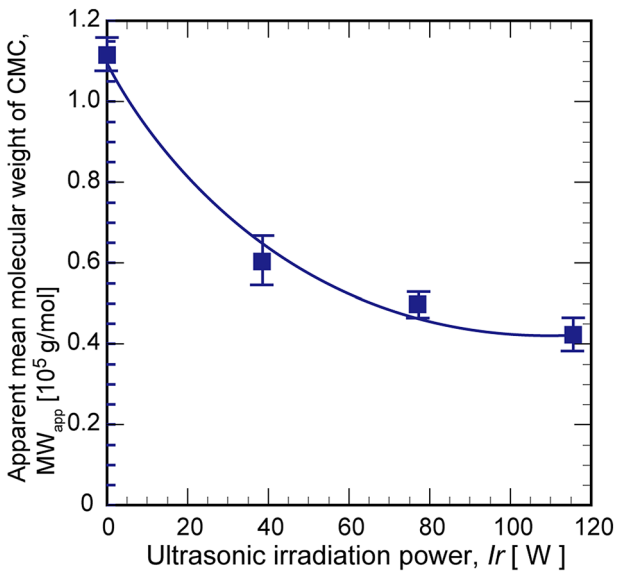

Fig. 5 Effect of apparent mean molecular weight on ultrasonic irradiation power measured by the Mark–Houwink–Sakurada equation

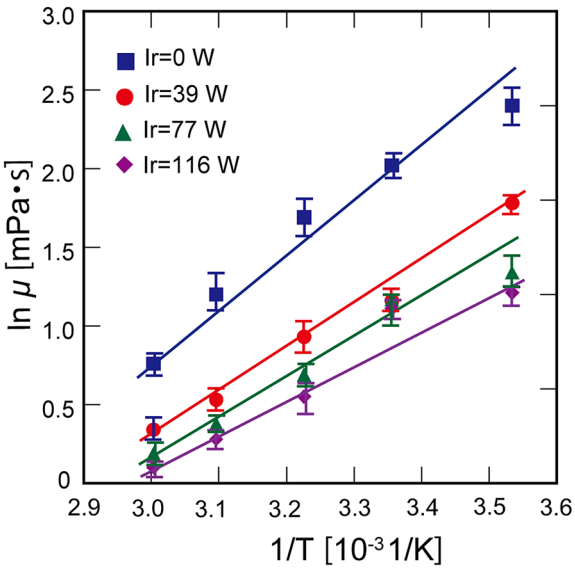

Fig. 6 Viscosity of CMC plotted against reciprocal temperature

of cavitation bubbles also increases, giving a stronger cavitation effect and producing higher shear forces on collapse. It has been reported that the viscosity of an HDPE/Illite solution decreased with increasing ultrasonic power [14].

The temperature dependence of the viscosity of a liquid is given by the Andrade equation [15]:

$$\mu = A \cdot e^{\frac{E}{RT}} \quad (5)$$

$$\ln \mu = \ln A + E/(R \cdot T) \quad (6)$$

where A (Pa s) is the frequency factor, E (J/mol) is the activation energy, R is the gas constant [8.3 J/(mol K)], and T (K) is the heating temperature.

Figure 7 shows that the temperature dependence of the viscosity of the CMC solution closely follows the Andrade equation. The frequency and activation energy are deduced from the intercept and slope of this plot, respectively. The activation energy (E) decreases with increasing ultrasonic power. Figure 7a shows that ultrasound significantly decreases the energy barrier necessary for the reaction. The value of E with ultrasonic pretreatment at 116 W was 0.7 times the value in the untreated system. In this figure, the value of E is similar to the energy of a hydrogen bond (5–30 kJ) [16].

The energy input form ultrasonication was 209 kJ (= 116 W × 1800 s). Part of this energy influences the reduction of hydrogen bonding. The value of E represents the energy between the molecular chains necessary for the movement of the molecules in the liquid. When E is high, the intermolecular forces between the molecular chains

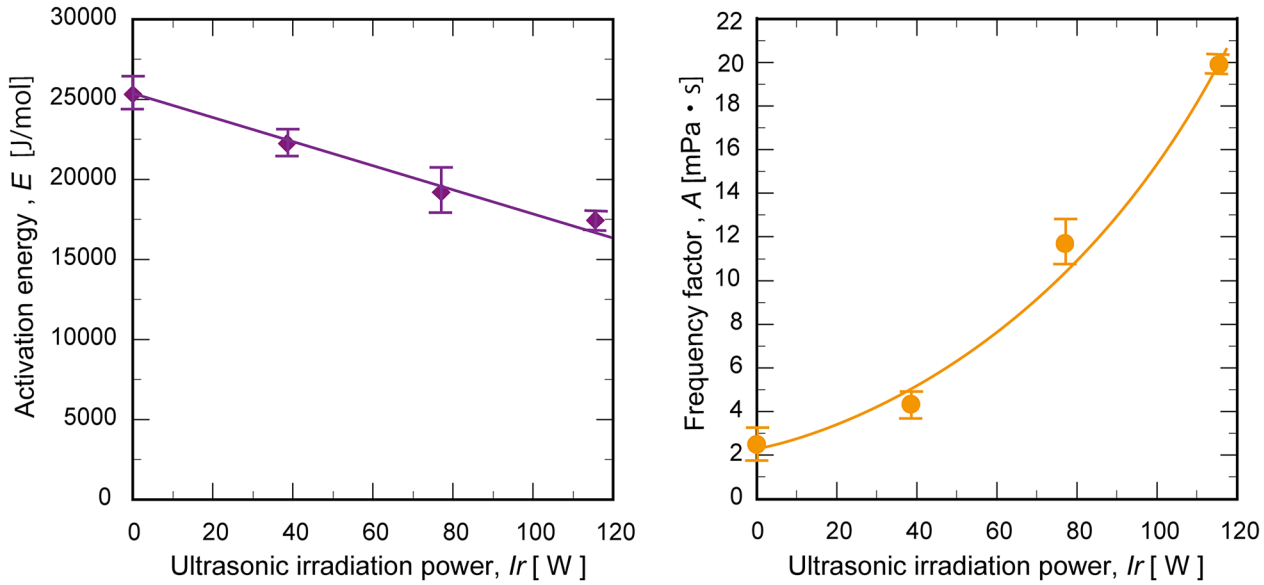

Fig. 7 Effect of ultrasonic irradiation power on activation energy and frequency factor calculated from Fig. 6

are large. Macromolecular chains, e.g., proteins and fats, tend to become entangled [17]. The frequency factor (*A*) increases with ultrasonic irradiation (Fig. 7b). Here, *A* represents the degree of free volume in which the molecule can flow. Therefore, the reduction in *E* and the increase in *A* due to ultrasonic irradiation are attributed to the decrease in the intermolecular forces in the CMC molecular chains, disentangling them and increasing the space in which the molecules can move.

4 Conclusion

We successfully demonstrated control of the viscosity of water-soluble cellulose and the enhancement of its enzymatic degradation rate using ultrasonication. Ultrasonication increased glucose production in the system employing *A. niger*. The initial reaction rate V_i of the system employing *A. niger* was 1.5 times higher than that of the system employing *T. reesei*. The kinetic parameters of enzymatic hydrolysis were influenced by the ultrasonic pretreatment. The Michaelis constant K_m was reduced to 0.2 times its original value in the system employing *A. niger* and 0.6 times its original value in that employing *T. reesei*. The maximum reaction rate V_{max} slightly increased in both systems.With this approach, both the reaction rate and yield increased in the system employing *A. niger*. These results can be explained based on the different hydrolysis reaction mechanisms of *A. niger* and *T. reesei*.

The viscosity of the CMC solution and the apparent mean molecular weight of CMC decreased with increasing ultrasonic irradiation power. Ultrasonic irradiation clearly reduced the viscosity, and the degree of this reduction depended on the concentration of the solution. With increasing ultrasonic irradiation power, the activation energy decreased, and the frequency factor increased. Ultrasonic irradiation can therefore be employed in process design to control the viscosity of these solutions and achieve high-performance enzymatic hydrolysis of water-soluble cellulose.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

1. Xuliang L, Cheng C, Hongming L, Xueing Q, Yuxia P, Dongjie Y (2017) Effect of cationic surfactant cetyltrimethylammonium bromide on the enzymatic hydrolysis of cellulose. Cellulose 24:61–68
2. Xuliang L, Linjun W, Siqi H, Yanlin Q, Xueqing Q, Hongming L (2019) Effect of lignin-based amphiphilic polymers on the cellulase adsorption and enzymatic hydrolysis kinetics of cellulose. Carbohydr Polym 207:52–58
3. Xuliang L, Yaosen Y, Lei W, Linjun W, Danyuan X, Yanlin Q (2019) Improved enzymatic hydrolysis of hardwood and cellulase stability by biomass kraft lignin-based polyoxyethylene ether. Int J Biol Macromol 136:540–546

4. Hatanaka D, Yamaoto K, Kadokawa J (2014) Preparation of chitin nanofiber-reinforced carboxymethyl cellulose films. Int J Biol Macromol 69:35–38
5. Prajapat AL, Das P, Gogate PR (2016) A novel approach for intensification of enzymatic depolymerization of carboxymethyl cellulose using ultrasonic and ultraviolet irradiations. Chem Eng J 290:391–399
6. Suslick KS (1990) Sonochemistry. Science 247:1439–1445
7. Alvira P, Pejo ET, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101:4851–4861
8. Sulaiman AZ, Ajit A, Chisti Y (2013) Ultrasound mediated enzymatic hydrolysis of cellulose and carboxymethyl cellulose. Biotechnol Prog 29:1448–1456
9. Sakka K, Okada H (2008) Cellulolytic systems of microorganisms. Cellul Commun 15:104–108 **(in Japanese)**
10. Kulicke WM, Clasen C, Lohman C (2005) Characterization of water-soluble cellulose derivatives in terms of the molar mass and particle size as well as their distribution. Macromol Symp 223:151–174
11. Chan R (2004) Physical chemistry for the bioscience. University Science Book, Mill Valley
12. Gogate PR, Pandit AB (2004) Sonochemical reactors: scale up aspects. Ultrason Sonochem 11:105–117
13. Mohod AV, Gogate PR (2011) Ultrasonic degradation of polymer: effect of operating parameters and intensification using additives for carboxymethyl cellulose (CMC) and polyvinyl alcohol (PVA). Ultrason Sonochem 18:727–734
14. Li Y, Chen G, Guo S, Li H (2005) Effect of ultrasonic oscillations on the rheological behavior and morphology of illite-filled high-density polyethylene composites. J Appl Polym Sci 96:379–384
15. Diaz RM, Bernardo MI, Fernandez AM, Folgueras MB (1996) Prediction of the viscosity of lubrication blends at any temperature. Fuel 75:574–578
16. Chang R (2004) Physical chemistry for the bioscience. University Science Book, Mill Valley
17. Bloore CG, Boag IF (1981) Some factors affecting the viscosity of concentrated skim milk. NZ J Dairy Sci Technol 16:143–154